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應用改良的蛋白質體質譜法來探討錳離子對耐輻射奇異球菌 (Deinococcus radiodurans)生長的影響
研究成果報告(精簡版)

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計畫參與人員：碩士班研究生-兼任助理：翁嘉嬋
專題生：洪千喬、許尹鐘、黃家祥

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Using high-concentration trypsin-immobilized magnetic nanoparticles for rapid *in situ* protein digestion at elevated temperature

Jingyueh Jeng[1]*, Mon-Fang Lin[2], Fong-Yu Cheng[3], Chen-Sheng Yeh[3] and Jentaie Shiea[2,4]

[1]Department of Biotechnology, Chia Nan University of Pharmacy & Science, 60, Erh-Jen RD., Sec.1, Jen-Te, Tainan 717, Taiwan
[2]Department of Chemistry, National Sun Yat-Sen University, 70 Lien-hai Road, Kaohsiung 804, Taiwan
[3]Department of Chemistry, National Cheng-Kung University, 1 Nuiversity Road, Tainan 701, Taiwan
[4]National Sun Yat-Sen University-Kaohsiung Medical University Joint Center, 70 Lien-hai Road, Kaohsiung 804, Taiwan

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We describe an innovative approach – using a high concentration of trypsin-modified magnetic nanoparticles (TMNPs) – for the rapid and efficient digestion of proteins at elevated temperature. The required digestion time could be reduced to less than 10 s. After digestion, the TMNPs were collected magnetically from the sample solution for reuse and the digested peptides were characterized using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry. Protein digestion was optimized when using the TMNPs (5 μg/μL) at 57°C; a significantly high peptide coverage was achieved for protein identification (e.g., 98% for lysozyme). Although a high concentration of TMNPs was used for digestion, the short digestion time led to much lower amounts of trypsin peptides being produced through self-digestion. As a result, interference in the mass spectrometric detection of the peptide ions was reduced significantly. Copyright © 2007 John Wiley & Sons, Ltd.

In modern proteomics, efficient proteolytic digestion of proteins is a critical step toward their identification. Solution-based digestion processes are generally less time-consuming and provide more-complete protein coverage than do in-gel digestions. Traditional solution-based protein digestion methods, however, present a number of problems that may limit the advancement of high-throughput protein identification technologies. For example, the long digestion times required because low protease-to-substrate ratios must be maintained to avoid the interference of autolysis peptides lead to the formation of 60–70% peptide sequence coverage obtained for standard proteins. In an effort to circumvent the drawbacks of proteolytic digestion – usually using trypsin – alternative proteolytic digestion procedures have been developed recently to accelerate the digestion procedure. Because the enzyme concentration and the temperature affect the rate of digestion, enhanced digestion rates are obtained when confining the reactions to small volumes at elevated temperature. These conditions can be achieved when increasing the concentration of trypsin,[1] lifting the protein temperature prior to tryptic digestion,[2–4] and raising the temperature of the proteolytic reaction using a water bath[5,6] or microwave heating system.[7–9] Microwave-assisted digestion of proteins in solution or in a gel occurs within minutes.[7] Furthermore, enzymatic cleavage of proteins can be enhanced in an ultrasonic field, reducing the sample handling time from hours to seconds.[10] A similar enhancement occurs in aqueous organic systems, which aid the in-solution digestion of soluble, protease-resistant proteins.[11–13] This phenomenon arises because proteins tend to denature in the presence of organic solvents, such as acetonitrile, thereby increasing the enzyme's accessibility to the cleavage site. Other approaches that mimic enzymatic digestion through microwave-based acid-catalyzed hydrolysis have been developed as an alternative technique to degrade proteins into small peptides for mass spectrometric (MS) analysis.[14–18]

Although significant progress has been made in the degradation of proteins, these methods all feature residual protease in the reaction mixture, which leads to a loss of sensitivity and also an increase in the complexity of the mass spectra, placing a constraint on the amount of sample that can be used. Additionally, digestion at elevated temperatures or under acidic conditions inevitably leads to a greater number of digestion artifacts, such as the products of transpeptidation, nonspecific cleavage, deamidation, oxidation, and protease self-digestion. These unwanted products can interfere seriously with matrix-assisted laser desorption/ionization (MALDI)-MS and electrospray ionization (ESI)-MS analyses.

**Correspondence to*: J. Jeng, Department of Biotechnology, Chia Nan University of Pharmacy & Science, 60, Erh-Jen RD., Sec.1, Jen-Te, Tainan 717, Taiwan.
E-mail: jyjeng@mail.nsysu.edu.tw
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Recently, high concentrations of trypsin immobilized through covalent bonding onto various solid supports (such as confined zones of capillaries,[19] packed beads,[20–26] porous silicon,[27] and porous polymer monoliths[28]) have been used to facilitate proteolysis with enhanced protease stability and reusability. Using immobilized trypsin can reduce the diffusional limitations experienced in solution-based hydrolyses, thus improving the degree of mass transfer and shortening the multi-hour conventional digestion procedure down to several minutes. Additional benefits of this approach are the high effective concentrations of the enzymes, reduced degrees of autolysis, and the ability to increase the degree of automation via continuous flow through a packed reactor bed.[29–33]

Although on-line digestion reactors are relatively fast and easy to automate, the increased complexity of such systems may result in a higher risk of peak broadening resulting from dead volumes, the need for an adequate sample volume for digestion, and the requirement of long incubation times in the digestion reactor to effect comprehensive digestion. Additionally, most available methods for protease immobilization require multistep reactions. Thus, there remains considerable scope for the development of simple and efficient digestion techniques. One of the most promising methods explored recently is the use of tailored magnetic nanoparticles for the separation of a series of binary and ternary protein mixtures.[34] Magnetic nanoparticles combined with high-gradient magnetic filtration provide a potentially effective means for the rapid recovery of microsolutes from suspensions of other colloidal materials.[35–37]

In this paper, we report the immobilization of trypsin onto magnetic nanoparticles (TMNPs) for the digestion of proteins. These loaded TMNPs could be separated effectively from multicomponent reaction mixtures by using an appropriately applied external magnetic field. We found that the use of these TMNPs (a) allowed high-temperature digestion to be performed without the need for sample pretreatment, (b) improved the digestion time to within a few seconds, and (c) reduced the sample volume down to several microliters.

EXPERIMENTAL

Materials and reagents

Cytochrome C, myoglobin (horse heart), lysozyme, human immunoglobin G (IgG), ammonium bicarbonate, α-cyano-4-hydroxycinnamic acid (CHCA), 2,5-dihydroxybenzoic acid (DHB), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), and trypsin (bovine pancreas) were purchased from Sigma, Taiwan. Human serum was from a volunteer.

Fe_3O_4 nanoparticles[38]

For the preparation of a dispersed Fe_3O_4 nanoparticle solution, a process of two-stage addition of a protective agent and chemical co-precipitation was employed. Briefly, two solutions containing Fe^{II} and Fe^{III} at a predetermined concentration ratio were mixed, followed by the addition of an organic acid as adherent. Subsequently, the pH of the solution was carefully adjusted using NaOH and a suitable amount of adherent was added to achieve complete coating of the particle surface such that the dispersed Fe_3O_4 nanoparticles were functionalized with $-NH_3^+$ groups.

Formation of Fe_3O_4/trypsin conjugates and measurement of enzymatic activity

A trypsin solution (0.5 mM, 60 μL) and EDC (0.1 mg) were added to an Fe_3O_4 dispersion (0.25 mM, 4 μL) mixed with phosphate buffer (pH 8, 10 mM, 434 μL). The solution was incubated for 4 h at 4°C. Free trypsin remaining in the supernatant was removed. The powder obtained was rinsed and re-suspended in sodium phosphate buffer solution (pH 8) for enzyme activity assays.[39] The conjugation efficiency was estimated by measuring the UV absorption of the aromatic units at 280 nm (i.e., for π–π^* transitions in tryptophan and tyrosine residues) before and after the addition of trypsin into the dispersion of Fe_3O_4 nanoparticles.

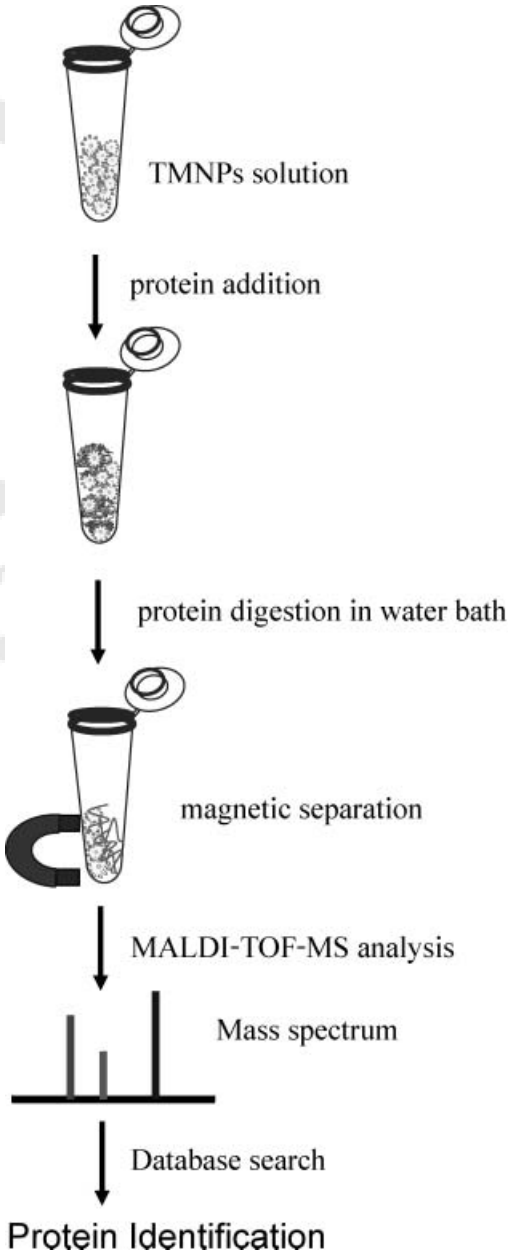

Figure 1. Schematic illustration of protein digestion performed using trypsin-immobilized magnetic nanoparticles in a water bath.

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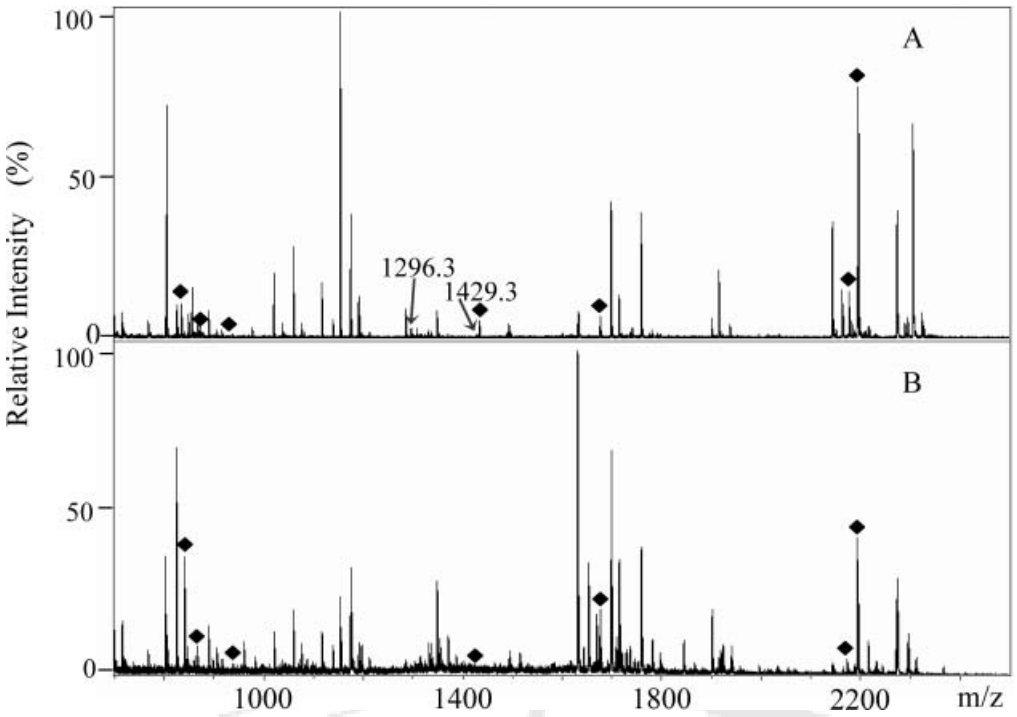

Figure 2. MALDI-TOF mass spectra of the peptides generated from 1 μg/μL trypsin (2 μL) in (A) the presence and (B) the absence of 10^{-6} M lysozyme (2 μL), after in-solution digestion at 37°C in a water bath for 10 min. Diamond-shaped symbols denote the proteolytic peptides of the lysozyme that overlap with the peptides from trypsin autolysis.

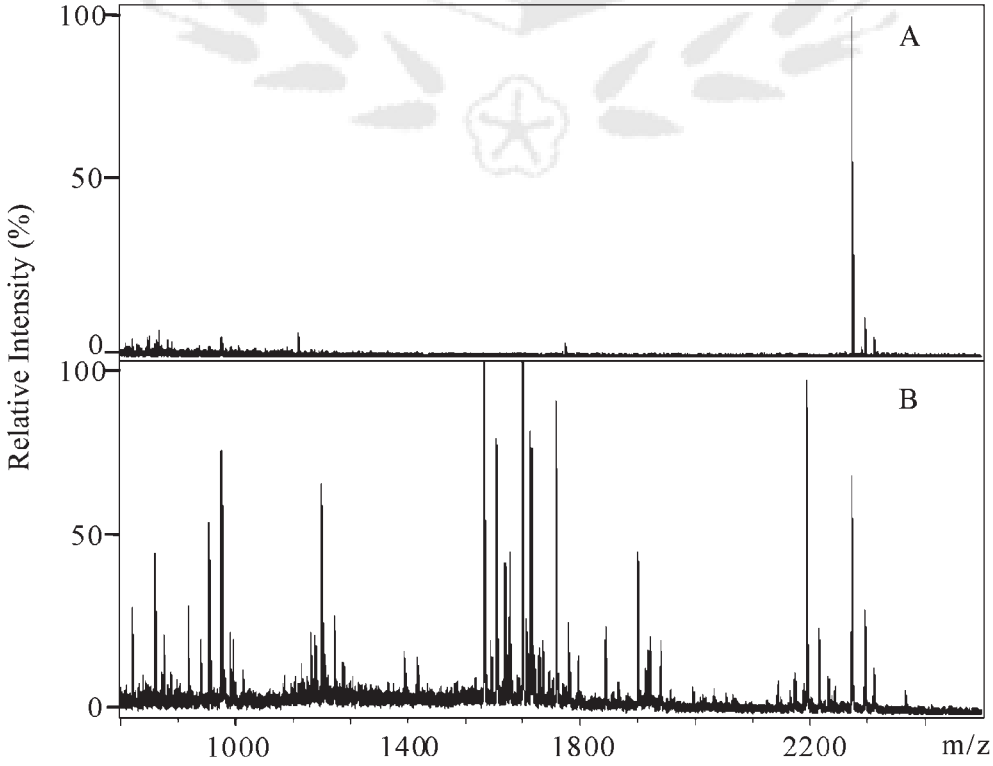

Figure 3. MALDI-TOF mass spectra of the peptides generated from (A) 1 μg/μL TMNPs and (B) 1 μg/μL trypsin after digestion at 37°C in a water bath for 10 min.

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Protein digestion on TMNPs

A protein sample (5 μL) was added to the suspension of TMNPs (5 μL of the 5 μg/μL re-suspension in 50 mM NH_4HCO_3). The digestions were performed at either 37 or 57°C for 0.5, 1, 3, 5, or 10 min (Fig. 1). An aliquot (1 μL) of each sample was removed for mass spectrometric analysis. A database search was performed using the Mascot program.

RESULTS AND DISCUSSION

The digestion rates of proteins are significantly accelerated when using a high concentration of trypsin without performing reductive alkylation of the proteins, although this does not reflect on the completeness of the cleavage. Figure 2 illustrates the mass spectra obtained after using 1 μg/μL trypsin for solution digestion in the presence and absence of lysozyme (10^{-6} M) without any pretreatment at 37°C in a water bath for 10 min. A total of nine proteolytic peptides from lysozyme were observed against the Mascot database, but only two small peptides (*m/z* 1296.3 and 1429.3; Fig. 2(A)) were confirmed distinctly from a comparison with the mass spectrum of trypsin autolysis (Fig. 2(B)) because of overlap with peaks of the tryptic peptides. In addition, signals from autolysis of trypsin are likewise expected to dominate that of lysozyme by suppression effects in this experiment.

Therefore, we thought that the use of TMNPs would overcome such interference from tryptic digestion fragments – which, in most cases, occur in the mass range from 800 to 3000 Da – in protein digestion mixtures. The positive effects of protein digestion of randomly immobilized trypsin on the TMNPs were analyzed using mass spectrometry. Figure 3(A) displays the MALDI time-of-flight (TOF) mass spectra of the autolysis of the TMNPs, performed without any pretreatment at 37°C in a water bath for 10 min; we observe no complicating signals between *m/z* 1000 and 2500, i.e., the autolysis of TMNPs does not contribute to the appearance of any background peaks in this range, unlike the results of conventional in-solution enzyme digestion (cf. Fig. 3(B)). Although trypsin autolysis did generate several peptides in the mass range between 2250 and 2350 Da at a concentration of 1 μg/μL, they are unlikely to significantly interfere with database searching.

The typical steps of protein digestion involve denaturation, reduction, and alkylation, with incubation of the protein

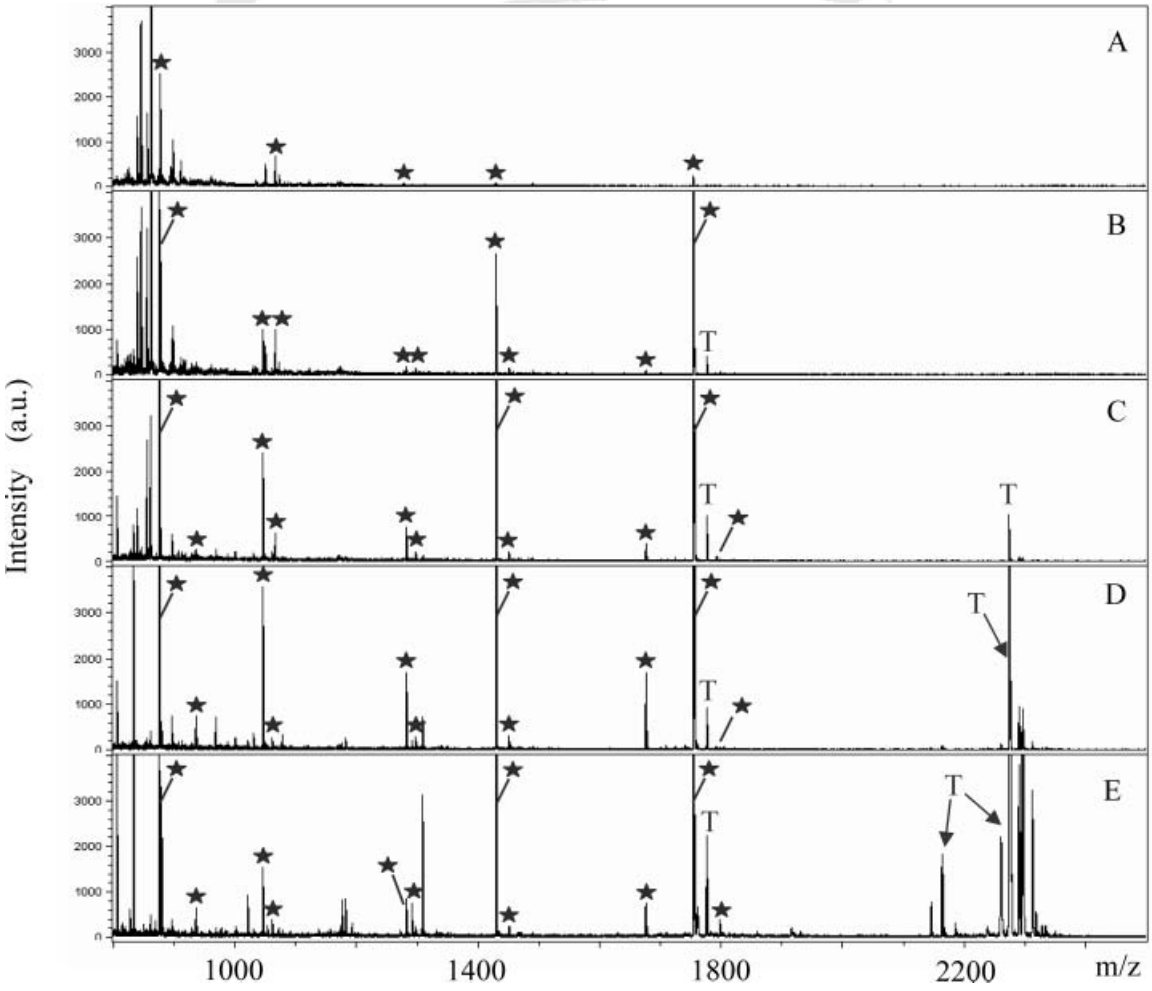

Figure 4. MALDI-TOF mass spectra of the peptides generated from 10^{-6} M lysozyme after digestion using (A) 0.1, (B) 1, (C) 5, (D) 10, and (E) 50 μg/μL TMNPs at 37°C in a water bath for 10 min; the TMNPs-to-protein ratios ranged from 7:1 to 3500:1 (w/w). To provide a better comparison of the results, the highest intensity scale in each of the plots was standardized. Asterisk denotes a proteolytic peptide from lysozyme; the letter 'T' indicates peptides derived from trypsin autolysis.

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with trypsin overnight at recommended protease-to-protein ratios from 1:200 to 1:20 (w/w). Here, to take advantage of the low interference of the TMNPs and to facilitate protein degradation, we used rather high reverse protease-to-protein ratios (from 7:1 to 3500:1, w/w) to accelerate the protein digestion; the proteins were not pretreated to denaturation, reduction, and alkylation prior to trypsin digestion. In a parallel set of experiments, we analyzed the influence of the concentration of TMNPs on the performance of the protein digestion. We compared the mass spectra obtained for the proteolytic peptides from digested 1 μM lysozyme at various concentrations of TMNPs. As expected, Fig. 4 displays an increase in the digestion efficiency upon increasing the concentration of the TMNPs.

Interestingly, the peptide fragments from lysozyme were predominant from digestions where the concentration of proteins was lower than that of TMNPs. As indicated in Fig. 4(A), when lysozyme was subjected to digestion at 37°C using 0.1 μg/μL TMNPs, five peptides of low intensities were identified after 10 min. We observed more peptides (nine peptides in Fig. 4(B), eleven peptides in Figs. 4(C)–4(E)) at higher concentrations of TMNPs. The protein scores against the Mascot search were 72 (Fig. 4(A)), 112 (Fig. 4(B)), and 179 (Figs. 4(C)–4(E)). In this example, the initial concentration of TMNPs had to be greater than 1 μg/μL to provide significant protein scores when using this method. Additionally, three preponderant proteolytic peptides (at m/z 874.5, 1428.8, and 1753.9) clearly arose upon increasing the concentration of TMNPs.

Furthermore, Figs. 4(D) and 4(E) indicate that the intensities of the autolysis peptides in the mass range m/z 2250–2350 increased upon increasing the concentration of TMNPs; signals in this range of molecular weights, however, rarely interfere with protein identification. Thus, the performance was enhanced when using higher concentrations of TMNPs for this digestion procedure. In Fig. 4(E), the relatively high TMNPs-to-protein ratio of 3500:1 (w/w) allowed significant protein identification from databases through peptide mass fingerprinting at a very low substrate concentration without any interference from the signals from

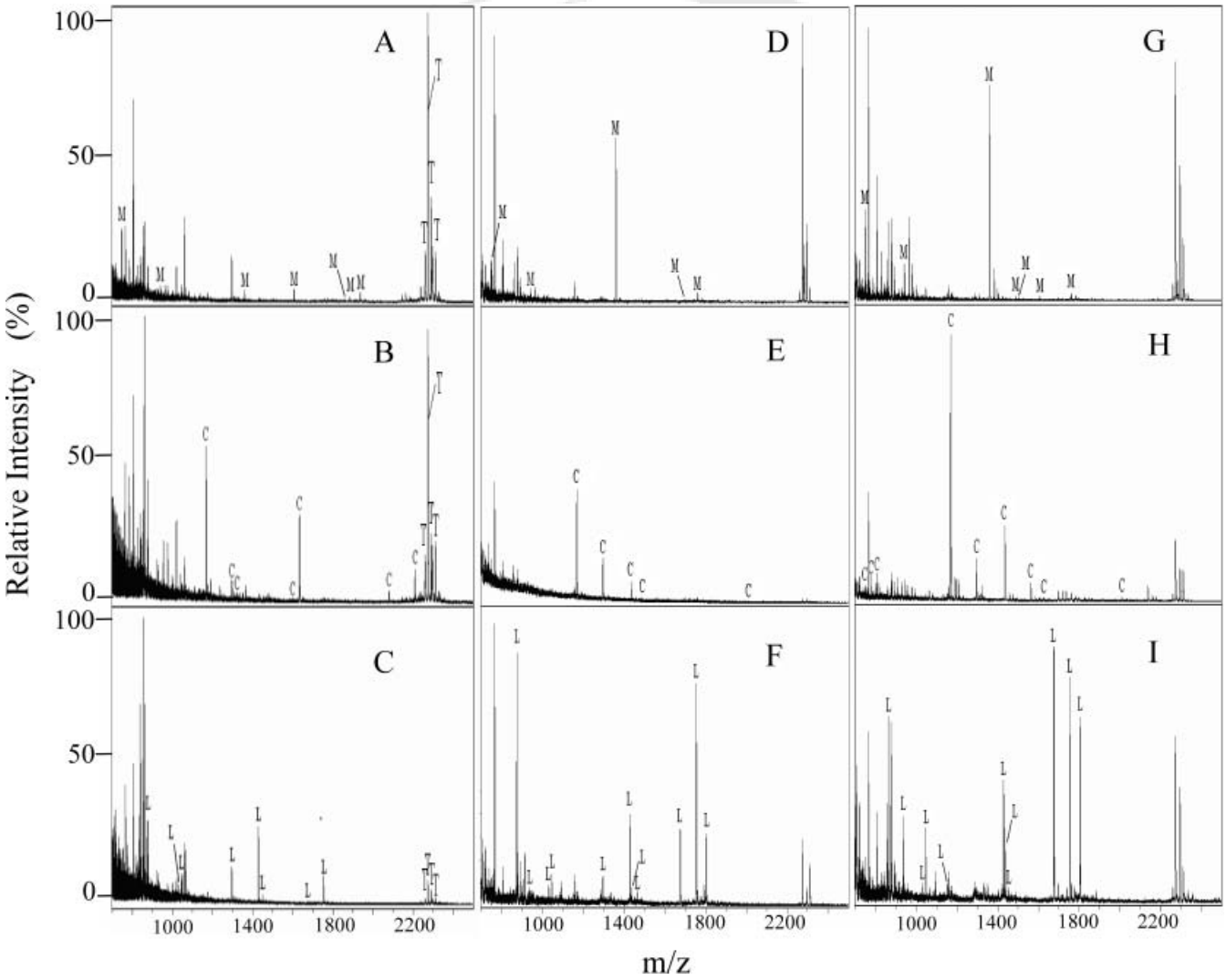

Figure 5. MALDI-TOF mass spectra of the peptides obtained after digestion using 5 μg/μL TMNPs (weight of trypsin/weight of substrate, 350:1) under various conditions. (A) Myoglobin, (B) cytochrome c and (C) lysozyme were digested at 37°C for 10 min; (D) myoglobin, (E) cytochrome c and (F) lysozyme were digested at 57°C for 30 s; (G) myoglobin, (H) cytochrome c and (I) lysozyme were digested at 57°C for 1 min. The digestion was performed in 12.5 mM ammonium bicarbonate. For better comparison of the results, the intensity scale in each plot was set to the intensity of the highest peak.

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Table 1. Summary of MALDI-TOF MS results obtained from digests by trypsin-immobilized magnetic nanoparticles

Sample	Myoglobin (in Fig. 5(G))	Cytochrome c (in Fig. 5(H))	Lysozyme (in Fig. 5(I))	Human IgG	Human serum
Peptides matched	7	10	11	2	17
Amino acid sequence coverage, %	32	49	57	21	29 (serum albumin)
Protein score	84	124	170	34	56
Digestion time	1 min at 57°C	1 min at 57°C	1 min at 57°C	3 h at 37°C	3 hr at 37°C

	Peptide sequence[a]				
Sample	Myoglobin	Cytochrome c	Lysozyme	Human IgG	Human serum
	135–140 ALELFR	9–14 KIFVQK	33–39 HGLDNYR	69–80 SSPFTFGQGTKL	1–19 MKWVTFISLLFLFSSAYSR
	147–154 YKELGFQG	81–87 MIFAGIK	80–86 WWCNDGR	54–66 VEPEFAVYYCQR	2–19 KWVTFISLLFLFSSAYSR
	135–146 ALELFRNDIAAK	74–80 KYIPGTK	32–39 RHGLDNYR		35–44 FKDLGEENFK
	120–134 HPGDFGADAQGAMTK	81–88 MIFAGIKK	135–143 GTDVQAWIR		66–75 LVNEVTEFAK
	141–154 NDIAAKYKELGFQG	29–39 TGPNLHGFGR	20–31 VFGRCELAAAMK		131–138 DDNPNLPR
	18–32 VEADIAGHGQEVLIR	29–40 TGPNLHGLFGRK	52–63 FESNFNTQATNR		139–160 LVRPEVDVMCTAFHDNEETFLK
	135–148 ALELFRNDIAAKYK	27–39 HKTGPNLHGLFGR	80–91 WWCNDGRTPGSR		162–168 YLYEIAR
		27–40 KTGPNLHGLFGRI	20–32 VFGRCELAAAMKR		162–169 YLYEIARR
		62–74 EETLMEYENPKK	116–130 IVSDGNGMNAWVAWR		170–183 HPYFYAPELLFFAK
		57–73 GITWKEETLMEYLE	64–79 NTDGSTDYGILQINSR		237–242 AWAVAR
			115–130 KIVSDGNGMNAWVAWR		362–372 HPDYSVVLLLR
					362–375 HPDYSVVLLLRLAK
					397–413 VFDEFKPLVEEPQNLIK
					427–434 FQNALLVR
					439–452 VPQVSTPTLVEVSR
					525–543 EFNAETFTFHADICTLSEK
					589–597 ETCFAEEGK

[a] Mascot program was used to identify digest fragments. The protein databases Swiss Prot and NCBI were used. Trypsin was chosen as the digestion enzyme. All other possible modifications, such as an alkylation of the cysteine residue, were not considered. Mass tolerance or mass error range was kept below 1.0 Da throughout the entire protein search.

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the autolysis of trypsin. Consistent with the observed tryptic digestion, we confirmed the products derived from a lysozyme through subsequent database searching (first ranking; protein scores, 179; sequence coverage, 67%; allow maximum two missed cleavages), but not all of the expected peptides were found in the spectra because of incomplete enzymatic digestion during such a short digestion time (10 min). Therefore, the digest fragments present in the MALDI-TOF mass spectra were identified using the Mascot program and significant protein scores were given without MS/MS experiments for standard proteins. Additionally, we found that 5 μg/μL of TMNPs provided suitable peptide mass mapping without a severe ion suppression effect. Thus, we selected the 5 μg/μL suspension of TMNPs for subsequent digestion experiments.

Applying elevated temperatures is a simple means for dissociating protein aggregates; in addition, it can be used to further improve the efficiency and yield of protein digestion. Thus, we used MALDI-MS peptide data to study the digestion efficiency of proteins mediated by our immobilized trypsin at elevated temperatures (37, 57, and 77°C). To determine the optimum time required to obtain a reproducible map, we varied the digestion period from 30 s to 1 min. Studies were conducted to compare the extent of tryptic peptide generation from three proteins – myoglobin, cytochrome c, and lysozyme – each at a concentration of 10^{-6} M. Comparable signal-to-noise ratios and intensities were observed for the three systems, but the abundance of peptide fragments of digested lysozyme (Fig. 5) was much greater than those from cytochrome c and myoglobin, which were similar to those observed under the otherwise conventional digestion conditions. Importantly, we observed a higher abundance of peptide fragments from the digestion of TMNPs at 57°C relative to that at 37°C. Thermal treatment at 57°C allowed the protein digestion time to be shortened to 30 s while still generating sufficient peptide fragments for database searching. When the digestion time was extended to 1 min (Figs. 5(G)–5(I)), the intensities of the peptide fragments of the three proteins rose, but we observed only one or two extra fragments in the peptide mapping. Thus, there was no significant difference between the digestions performed for 30 s and 1 min; i.e., a 30-s digestion time is satisfactory for rapidly digesting the proteins and identifying them in this system. Table 1 lists the results of protein database searches from the above three MALDI mass spectra (Figs. 5(G)–5(I)).

It is clear that thermal TMNP-mediated digestion is a simple and effective strategy generating mass spectra that consist of a few peaks that can be readily searched using the Mascot program. We obtained poorer digestion results, however, when we performed the reaction at 77°C. One probable explanation is that the TMNPs were destabilized at

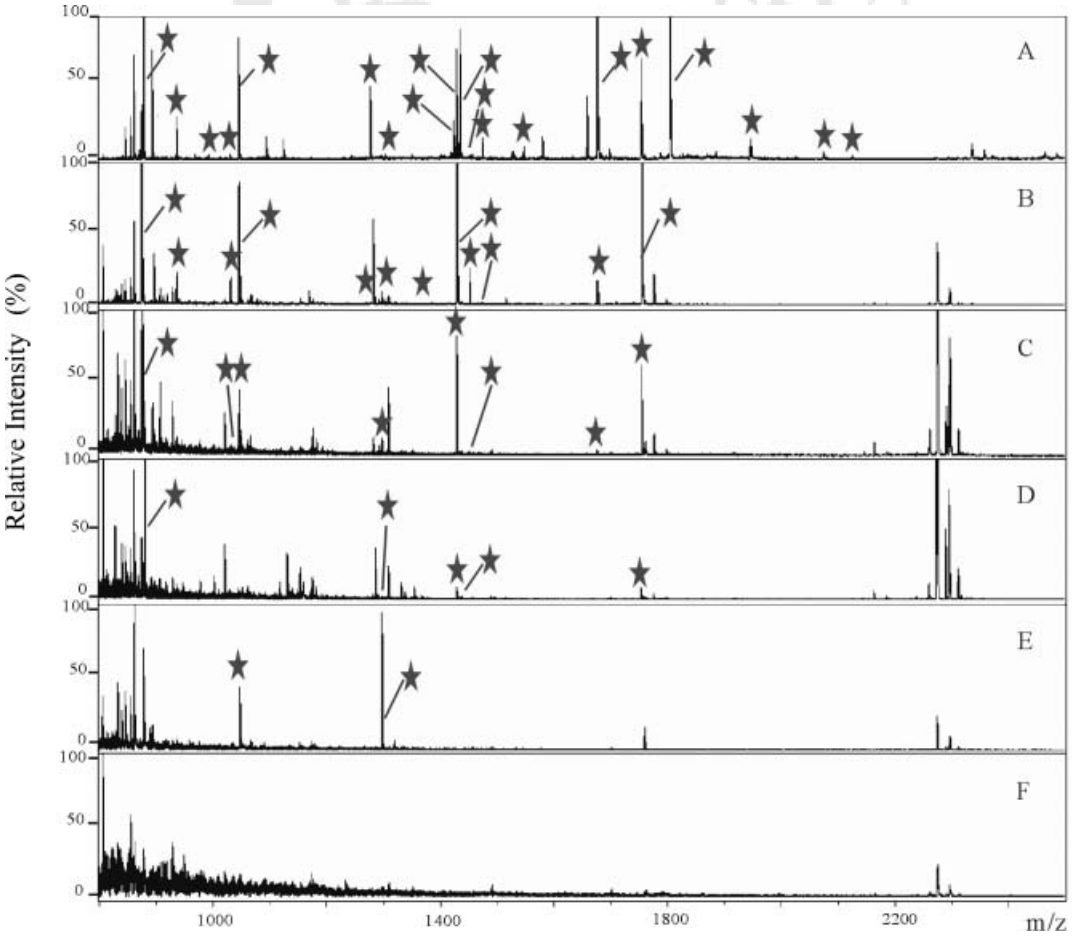

Figure 6. MALDI-TOF mass spectra of the peptides generated from (A) 10^{-5}, (B) 10^{-6}, (C) 10^{-7}, (D) 10^{-8}, (E) 10^{-9}, and (F) 10^{-10} M lysozyme through digestion using 5 μg/μL TMNPs at 57°C in a water bath for 1 min. Asterisks denote a proteolytic peptide from lysozyme.

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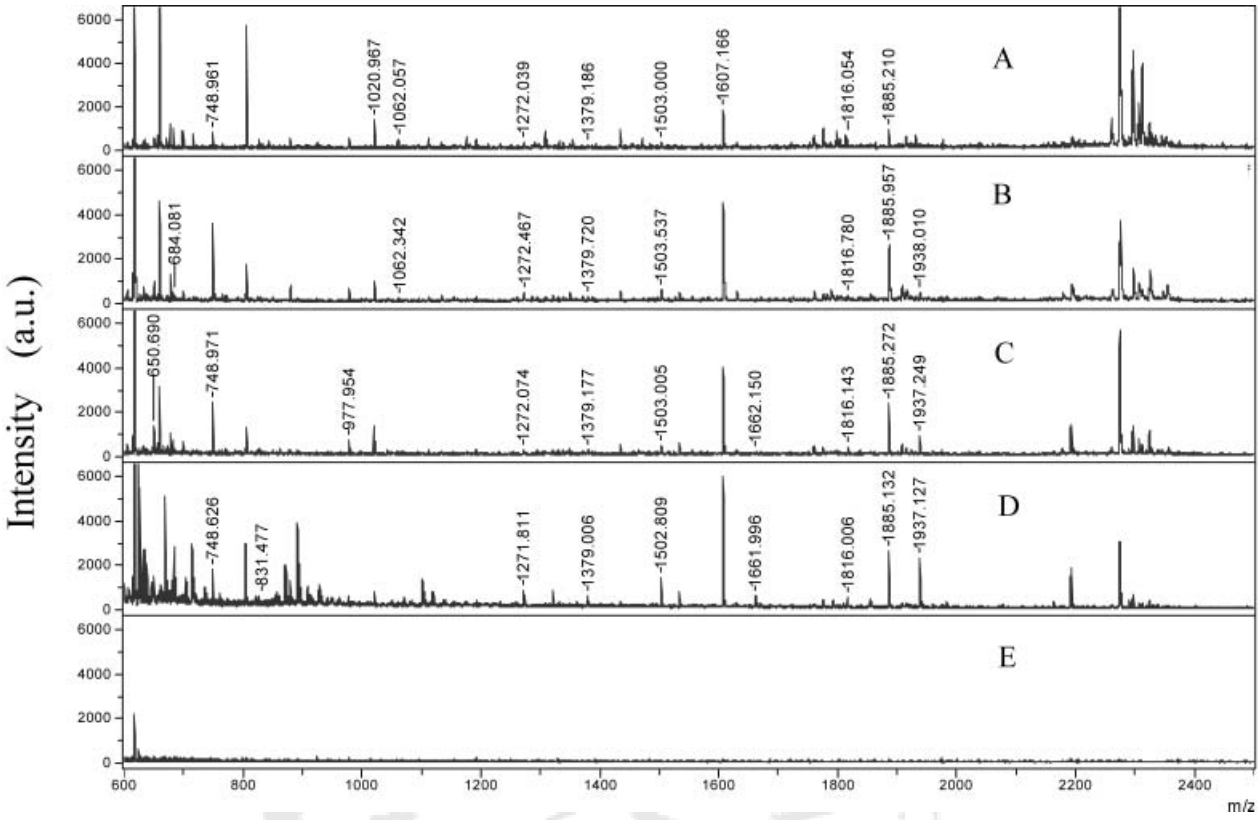

Figure 7. MALDI-TOF mass spectra of the tryptic digest product of myoglobin obtained by repeated use of TMNPs under water bath heating at 37°C for 10 min. The cycle number is (A) 1 time, (B) 3 times, (C) 6 times, (D) 9 times, and (E) 10 times. Each number denotes a proteolytic peptide from myoglobin.

this temperature, leading to an increase in the amount of autolysis (data not shown). To further evaluate the digestion of TMNPs, we analyzed two samples of human immunoglobin G (IgG) and human serum (10-fold dilution). Both these two samples were hardly digested after 3 h of incubation with TMNPs at 37°C. Two peptide fragments (*m*/*z* 1269.168 and 1618.638, 2 out of 7 peptides matched, sequence coverage 21%, score = 34, protein matched to human immunoglobin heavy chain FaB fragment) were found for human IgG digestion. Human serum exhibits a very similar digestion pattern to human serum albumin (17 out of 44 peptides matched, sequence coverage 29%, score = 56, protein matched to human serum albumin), i.e., the signals of proteolytic peptides from low-abundance proteins were suppressed in the presence of high-abundance proteins (for more details of peptide fragments, see Table 1).

For the detection limit of this rapid digestion technique, we performed target protein concentration studies over five orders of magnitude while maintaining the concentration of TMNPs constant. Figure 6 depicts the MALDI mass spectra obtained after the efficient high-speed digestion of small sample volumes (1–2 μL) of lysozyme in the sub-attomole range (from 10 pmol to 100 amol, i.e., concentrations from 10 μM to 100 pM). The number of fragments clearly decreased upon decreasing the concentration of the protein. We detected a maximum of 19 peptides from the digestion of 10^{-5} M lysozyme, resulting in a sequence coverage of 98% and a protein score of 272 (Fig. 6(A)). In Fig. 6(D), the digestion of 1 μL of a solution of 10 nM lysozyme yielded five recognized peptide fragments in the subsequent MALDI-MS analysis, giving a sequence coverage of 46% and a protein score of 75. This decreased number of peptide fragments and lower protein score did not affect the results of the Mascot search, which indicated the top score for (chicken) lysozyme. There were only two peptide fragments observed from the protein digestion (Fig. 6(E)), and none at the lowest-concentration (100 amol) protein digestion (Fig. 6(F)). Thus, this thermal TMNP digestion approach is capable of identifying standard protein samples at abundances as low as 10 fmol (i.e., a concentration of 10 nM). The mass spectra of the reused digestion of TMNPs were recorded under water bath heating (37°C) for 10 min. The numbers of peptide fragments did not decrease clearly from Fig. 7(A) to 7(D). It is seen that the decrease in the intensity of the tryptic peptide (*m*/*z* 2100–2400) results from an increase in the cycle number of repeated use of TMNPs (about 9 times) in Fig. 7. These results led to the signals from myoglobin being dominated gradually without the suppression effect.

CONCLUSIONS

This microscale thermal-TMNPs digestion method for protein identification decreases the digestion time to only 30 s, reduces the extent of enzyme autolysis, and improves the spectral quality. These results demonstrate how the analysis and identification of proteins can be improved when they are digested using a high-concentration enzyme immobilized on a solid support under appropriate thermal

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environmental conditions. Importantly, the target proteins do not need to be chemically denatured prior to analysis. The digestion of lysozyme led to the identification of 19 proteolytic peptides representing a sequence coverage of 98%; traditional methods yield the same peptides, but only after a prolonged digestion time. In addition, these TMNPs are readily collected for reuse by using an external magnet, and the cartridge can be cleaned by using a flow injection system.

Acknowledgements

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REFERENCES

1. Harris WA, Reilly JP. *Anal. Chem.* 2002; **74**: 4410.
2. Semino GA, Cerletti P. *J. Agric. Food Chem.* 1987; **35**: 656.
3. Park ZY, Russell DH. *Anal. Chem.* 2000; **72**: 2667.
4. Park ZY, Russell DH. *Anal. Chem.* 2001; **73**: 2558.
5. Havlis J, Thomas H, Sebela M, Shevchenko A. *Anal. Chem.* 2003; **75**: 1300.
6. Bark SJ, Muster N, Yates JR III, Siuzdak G. *J. Am. Chem. Soc.* 2001; **123**: 1774.
7. Pramanik BN, Mirza UA, Ing YH, Bartner PL, Weber P, Bose AK. *Protein Sci.* 2002; **11**: 2676.
8. Lin SS, Wu CH, Sun MC, Sun CM, Ho YP. *J. Am. Soc. Mass Spectrom.* 2005; **16**: 581.
9. Sun W, Gao SJ, Wang LJ, Chen Y, Wu SZ, Wang XR, Zheng DX, Gao YH. *Mol. Cell. Proteomics* 2006; **5**: 769.
10. Ferrer DLF, Capelo JL, Vazquez J. *J. Proteome. Res.* 2005; **4**: 1569.
11. Welinder KG. *Anal. Biochem.* 1988; **174**: 54.
12. Russell WK, Park ZY, Russell DH. *Anal. Chem.* 2001; **73**: 2682.
13. Slysz WG, Schriemer DC. *Rapid Commun. Mass Spectrom.* 2003; **17**: 1044.
14. Li AQ, Sowder RC, Henderson LE, Moore SP, Garfinkel DJ, Fisher RJ. *Anal. Chem.* 2001; **73**: 5395.
15. Zhong HY, Zhang Y, Wen ZH, Li L. *Nat. Biotechnol.* 2004; **22**: 1291.
16. Zhong HY, Marcus SL, Li L. *J. Am. Soc. Mass Spectrom.* 2005; **16**: 471.
17. Hua L, Low TY, Sze SK. *Proteomics* 2006; **6**: 586.
18. Swatkoski S, Russell SC, Edwards N, Fenselau C. *Anal. Chem.* 2006; **78**: 181.
19. Feng S, Ye ML, Jiang XG, Jin WH, Zou HF. *J. Proteome. Res.* 2006; **5**: 422.
20. Temporini C, Perani E, Mancini F, Bartolini M, Calleri E, Lubda D, Felix G, Andrisano V, Massolini G. *J. Chromatogr. A* 2006; **1120**: 121.
21. Turkova J, Kucerova Z, Benes MJ. *J. Mol. Recognit.* 1996; **6**: 360.
22. Yao ZP, Afonso C, Fenselau C. *Rapid Commun. Mass Spectrom.* 2002; **16**: 1953.
23. Warscheid B, Fenselau C. *Anal. Chem.* 2003; **75**: 5618.
24. Freije JR, Mulder PPMFA, Werkman W, Rieux L, Niederlander HAG, Verpoorte E, Bischoff R. *J. Proteome Res.* 2005; **4**: 1805.
25. Doucette A, Craft D, Li L. *Anal. Chem.* 2000; **72**: 3355.
26. Cooper JW, Chen JZ, Li Y, Lee CS. *Anal. Chem.* 2003; **75**: 1067.
27. Jang S, Kim D, Choi J, Row K, Ahn W. *J. Porous Mater.* 2006; **13**: 385.
28. Peterson DS, Rohr T, Svec F, Frechet JMJ. *J. Proteome Res.* 2002; **1**: 563.
29. Lippincott J, Hess E, Apostol I. *Anal. Biochem.* 1997; **252**: 314.
30. Kato M, Sakai-Kato K, Jin HM, Kubota K, Miyano H, Toyo'oka T, Dulay MT, Zare RN. *Anal. Chem.* 2004; **76**: 1896.
31. Calleri E, Temporini C, Perani E, De Palma A, Lubda D, Mellerio G, Sala A, Galliano M, Caccialanza G, Massolini G. *J. Proteome Res.* 2005; **4**: 481.
32. Slysz WG, Schriemer DC. *Anal. Chem.* 2005; **77**: 1572.
33. Khng HP, Cunliffe D, Davies S, Turner NA, Vulfson EN. *Biotechnol. Bioeng.* 1998; **60**: 419.
34. Krogh TN, Berg T, Hojrup P. *Anal. Biochem.* 1999; **274**: 153.
35. Luxton R, Badesha J, Kiely J, Hawkins P. *Anal. Chem.* 2004; **76**: 1715.
36. Slováková M, Minc N, Bílková Z, Smadja C, Faigle W, Futterer C, Taverna M, Viovy JL. *Lab Chip* 2005; **5**: 935.
37. Liu AL, Zhou T, He FY, Xu JJ, Lu Y, Chen HY, Xia XH. *Lab Chip* 2006; **6**: 811.
38. Chang FY, Yang YS, Huang WH, Yeh CS, Tsai CY, Wu CL, Shieh DB. *Biomaterials* 2005; **26**: 729.
39. Shieh DB, Chang FY, Su CH, Yeh CS, Wu MT, Tsai CY, Wu CL, Chen DH, Chou CH. *Biomaterials* 2005; **26**: 7183.

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出席國際學術會議心得報告

計畫編號	NSC 95-2113-M-041 -003
計畫名稱	應用改良的蛋白質體質譜法來探討錳離子對耐輻射奇異球菌 (*Deinococcus radiodurans*)生長的影響
出國人員姓名 服務機關及職稱	鄭淨月嘉南藥理科技大學生物科技系 副教授
會議時間地點	2007/6/3～6/07, 美國印第安那波里斯
會議名稱	第 55 屆美國質譜年會
發表論文題目	Detecting Organic Compounds Dissolved in Volatile Organic Solvents and Continuously Monitoring Chemical Reactions by Ambient Liquid Mass Spectrometry (ALMS)

一、參加會議經過

美國質譜學會 (American Society for Mass Spectrometry, ASMS)雖然是一個以美國為核心，結合美國與加拿大地區學術與實務界人士的專業學術團體，每年輪流在美加各大城市舉辦一次，但是會員來自世界各國，涵蓋學術及工業界，目前的會員超過7000位。主要的研究領域包括質譜技術開發、儀器的發展、及化學、地質、鑑識科學、生物及物理在質譜學上的應用。以其規模與悠久的歷史，及美國學界在世界上的優勢，ASMS所舉辦的研討會在國際上也佔了很重要的地位，也是全世界質譜學的學者、專家所共同參與的該領域中最大型的會議。

本屆會議於2007年6月3日至6月7日在美國印第安那波里斯市舉行。參加的人數達七千餘人，來自十多個國家。參加人員所提出的論文分別以專題演講、口頭報告及壁報討論等方式發表，在研討會期間，壁報論文(共2509篇)依其類別安排在不同時間張貼，作者需要在安排的時間內站在壁報旁解答讀者的問題。本次研討會將質譜技術分為五十六個主題包含質譜儀的儀器設計、質譜儀中離子反應及離子結構與能量探討;質譜於生物檢體的蛋白質影像分析、蛋白質體學分析、代謝體學分析、藥物代謝、核酸、高分子、刑事、醫學臨床的應用及高解析質譜儀質譜應用於蛋白質特性及環境偵測等等，其中還是以蛋白質體學(Proteomics)，生化學(Biochemistry)及製藥學(Pharmaceutics)有關之質譜分析研究為大宗。由於台灣近年來在質譜學研究方面質與量的長足進步，且連續幾屆均有不少學者與會，受到國際學界之肯定。這次台灣學術界參與此項會議的學者們總共發表了數十篇壁報論文，參與的學者及學生也將近50人左右。與會的亞洲國家(如日本，台灣，韓國，中國，及新加坡)也有逐漸增加的趨勢。

第一天研討會安排了報到及上課課程，由學有專精的各領域學者授課，但此課程需要收費。會議期間，大會每天上午08:00~08:45都安排了一場專題講座 (Plenary Lecture)，演講者均為各研究領域的佼佼者，其他各與會人士所提出的論文則分成口頭和壁報論文發表二種；與會人士可在任何時段自由選擇其中一組、聆聽報告及參與討論。壁報論文的討論 (Poster

Sessions) 則排在每天08:45~10:15及下午13:00~15:00之間進行。

由年會的論文發表的情形以及議程手冊可以看出，蛋白質影像分析、蛋白質體學分析及代謝體學分析等議題仍為今年較熱門的主題，幾乎有三分之一的論文均與蛋白質體學有關。此外最近發展出的大氣壓力游離質譜法因其方便性不需昂貴的真空幫浦，其發表的論文也形成另一熱門的主題。除了前述的演講與論文發表之外，大會另外安排了多場研習活動(Workshops and Interest Group Meetings)與參觀活動。

此外在會場中亦有不少與質譜學相關之專業書籍與文獻之展示販售或廣告，可依個人研究興趣購買或索取。

二、與會心得

每次參加美國質譜年會，籌備單位事前慎密之規劃使得研討會進行的可謂有條不紊，議程中亦甚少有調動或缺席之情況發生。除了經驗外，尚可歸功於籌備單位善用網路資源，將所有資料放在網路上，報名，繳交壁報論文，訂旅館，甚至oral section都可藉由網路聆聽，故大會進行的十分流暢順利。 每次大會亦舉辦數個小型討論會與論壇，開放與會人士自由發表意見並共同討論，經由討論都讓我受益良多，更加了解未來質譜學發展重要方向。 在與會的四天中，能與眾多相關領域的專家共同討論，相互提出見解與交換心得，令我覺得收獲頗豐。對於論文研究主題的進展與未來努力方向，也有更深刻的認識，同時也結交了些朋友，這對將來的研究會有實質的幫助。